

Reproducibility of prick skin tests to five common allergens

C. W. CLARKE, J. MITCHELL, A. J. NUNN* and J. PEPYS

Department of Clinical Immunology, Cardiothoracic Institute, London and

*M.R.C. Tuberculosis and Chest Diseases Unit, Brompton Hospital, London

(Received 29 May 1980; accepted for publication 7 June 1980)

Summary

Thirty-five asthmatic patients had prick skin tests to the common allergens *Candida albicans*, *Aspergillus fumigatus*, grass pollen, horse dander and *Dermatophagoides pteronyssinus* performed on a regular basis from Autumn 1973 to Autumn 1975. Specific IgE to the same allergens (except *C. albicans*) was determined at the time of skin testing for the first five seasons.

It was found that the position on the volar aspect of the forearm on which the test was performed did not affect the reaction. There was a significant variation in the percentage of patients with positive skin tests to *A. fumigatus*, grass pollen and horse dander with the latter showing a significant decrease with time. There was evidence of variation in weal size for all but *C. albicans*, and for grass pollen, horse dander and *D. pteronyssinus* there were reductions in weal size with time.

Significant differences were found for results of Log specific IgE for grass pollen and *D. pteronyssinus* over the study, but there was no trend. A good correlation between weal size and Log specific IgE for grass pollens and *D. pteronyssinus* was observed. For the four allergens, the coefficient of concordance between IgE levels within patients for the five seasons was highly significant.

Introduction

Prick skin tests have been shown to be a valuable supportive measure in the diagnosis and management of patients with atopic diseases (Pepys, Chan & Hargreave, 1968; McAllen, Assem & Maunsell, 1970; Stenius *et al.*, 1971; Hendrick *et al.*, 1975). Studies have indicated that there is a correlation between positive Type-I skin reactions to allergens demonstrated by prick testing and the presence of specific IgE to these allergens (Stenius *et al.*, 1971; Zetterstrom & Wide, 1974). It has also been demonstrated that the Type-I skin reaction is influenced by the atopic status of the patient (Pepys, 1973; Zetterstrom & Wide, 1974) and medications that the patient may be taking, e.g., antihistamines (Turner-Warwick, 1973).

Correspondence: C. W. Clarke, P. O. Box 310, Eastwood, 2122, N.S.W., Australia.

This paper presents the results of a study which set out to determine if weal size produced by skin prick testing with common allergens was:

- (a) influenced by the site on the arm the test was performed;
- (b) varied over time with repeat testing; and
- (c) related to specific IgE present at the time of testing to the same allergens.

Materials and methods

Patient selection

Thirty-five asthmatic patients being seen at regular intervals were selected for this investigation. The study covered the period from Autumn 1973 to Autumn 1975. Patients were skin tested at the commencement and then at regular intervals which corresponded to Winter 1973/1974, Spring 1974, Summer 1974, Autumn 1974, Spring 1975 and Autumn 1975. Patients on antihistamines were advised not to take these for 3 days prior to skin testing.

Allergens

The following allergens were used for skin testing: 150% horse hair and dander; 10% *C. albicans*; 1.2% *D. pteronyssinus*; 2.5% Group B2 pollens (grasses); 10% *A. fumigatus*; and Cocos solution (control).

Allergens were supplied by Bencard (Brentford, Middlesex) and the same batch was used for the duration of the study.

Allergen arrangement

Reading of the skin test weal size was conducted blind as follows. The Statistics Department of the Tuberculosis and Chest Diseases Unit (TCDU) prepared a schedule for the nurse technician performing the skin tests and a blank work sheet for the physician to record the weal sizes (Fig. 1). The left-hand side of the schedule corresponds to the offside arm position, the right-hand side to the nearside position. The numbering 1-6 corresponds to the numbers given to the control and five allergens

2	3	6
5	6	1
4	5	2
6	1	4
1	2	3
3	4	5

○	○	○
○	○	○
○	○	○
○	○	○
○	○	○
○	○	○

Fig. 1. The left-hand diagram is the worksheet prepared for the nurse technician detailing the antigen arrangement. The right-hand one is the blank worksheet used by the physician.

and the number code was kept constant throughout the study. Both sheets were returned to the TCDU for analysis.

Skin testing

Prick skin testing was performed according to the above schedule in a standard manner by nurse technicians trained for this purpose (Hendrick *et al.*, 1975). This involved three different people over the duration of the study.

The skin tests were read at 15 min. after the test was performed. These readings were performed blind as the physician was completely unaware of the order on the schedule used by the nurse technician. They were measured by the same person (C.W.C.) for the first five seasons. The diam. of the weal was measured with the use of a Bencard Gauge in mm and this was recorded on the above described form.

Specific IgE estimation

Blood was collected from each patient at each visit after the skin testing had been completed. Serum was then stored at -20°C and specific IgE's were estimated in one batch for each allergen for the first five seasons and in different batches for the remainder. Specific IgE was estimated by the RAST method (Wide, Bennich & Johansson, 1967). The same antigens used for the skin tests were coupled to the discs in the following concentrations: 10% *A. fumigatus*, diluted to 1/500; 2.5% grass pollen, diluted to 1/100; 1.2% *D. pteronyssinus*, diluted to 1/100; and 150% horse hair and dander, diluted to 1/5000.

The antigen of *C. albicans* was not thought to be suitable for use in this estimation. Control sera made up of cord sera and sera obtained from the blood bank were tested in duplicate. Only every fifteenth sera of the patients was tested in duplicate.

Allergen specific IgG estimation

IgG specific for *D. pteronyssinus* was estimated on sera collected at the beginning of the study and during the Autumn 1974 review, according to the method of Pepys *et al.* (1973).

Statistical methods

The results of the skin tests and the IgE determinations were analysed using analysis of variance techniques and correlations calculated using non-parametric correlation coefficients. The coefficient of concordance was calculated to investigate the consistency of the specific IgE determined on different occasions.

Results

Skin test data

Complete data for seven seasons from Autumn 1973 to Autumn 1975 inclusive was available for twenty-three patients. In none of the analyses was there any suggestion that position on the arm, nearside, centre or offside made any difference to the weal size ($P > 0.25$). The mean weal size and percentage of patients with positive results are shown in Table 1 for each allergen, season by season. There was a significant variation in the percentage of patients with positive skin tests to *A. fumigatus*, grass pollen and horse dander, the latter showing a significant decrease with time ($P < 0.001$). For grass pollen, horse dander and *D. pteronyssinus*, there were reductions in weal size with time

Table 1. Seasonal variation in weal size and skin test positivity (based on 23 patients with data for 7 seasons)

Allergen		Season (1973/1975)							Seasonal variation (P)	Trend
		Au	Wi	Sp	Su	Au	Sp	Au		
Control	Mean weal size (mm)	0.14	0.04	0.09	0.16	0.28	0.52	0.00	<0.001	N.S.
	% positive	4	1	4	6	9	10	0	<0.001	N.S.
<i>C. albicans</i>	Mean weal size (mm)	0.49	0.23	0.52	0.41	0.13	0.38	0.23	N.S.	N.S.
	% positive	13	7	14	12	4	10	7	N.S.	N.S.
<i>A. fumigatus</i>	Mean weal size (mm)	1.14	1.30	1.74	1.19	0.97	1.01	1.00	<0.001	N.S.
	% positive	19	16	29	16	19	20	13	<0.001	N.S.
Grass pollen	Mean weal size (mm)	4.10	3.29	2.65	2.88	3.04	2.14	2.45	<0.001	<0.05
	% positive	46	45	43	46	46	36	42	<0.001	N.S.
Horse dander	Mean weal size (mm)	2.90	2.72	2.43	2.57	2.25	2.42	1.68	<0.001	<0.01
	% positive	58	55	52	54	51	46	39	<0.001	<0.001
<i>D. pteronyssinus</i>	Mean weal size (mm)	4.41	4.30	4.19	4.13	3.55	3.61	3.80	<0.005	<0.05
	% positive	55	58	57	54	57	54	55	N.S.	N.S.

N.S. = Not significant.

Table 2. Seasonal variation in IgE (based on 35 patients with data for 5 seasons)

Allergen	Log _e IgE by season (1973/74)					Seasonal variation (P value)
	Au	Wi	Sp	Su	Au	
<i>A. fumigatus</i>	5.91	6.04	6.03	6.04	6.02	N.S.
Grass pollen	7.27	7.20	7.18	7.26	7.26	0.03
Horse dander	6.70	6.76	6.77	6.72	6.75	N.S.
<i>D. pteronyssinus</i>	7.26	7.30	7.22	7.13	7.22	0.03

N.S. = Not significant.

($P < 0.05$, < 0.01 , < 0.05 respectively for trend); for example, the mean weal size for *D. pteronyssinus* dropped from 4.41 mm in Autumn 1973 to 3.80 mm in Autumn 1975.

Specific IgE

Complete data was available on thirty-five patients for five seasons and Table 2 sets out the seasonal variation in the Log specific IgE values. Significant differences were found in the results for grass pollens and *D. pteronyssinus*, but there was no evidence of a trend with time. Table 3 shows the correlation between weal size and log specific IgE and it will be noted that the correlation was good for grass pollen and *D. pteronyssinus*, but was generally poor for *A. fumigatus* and horse dander.

For each of the four allergens, the coefficient of concordance (W) between the IgE trends within patients for the five seasons was highly significant ($P < 0.001$) but very

Table 3. A correlation between specific IgE and skin test positivity (thirty-five patients) (Kendall's τ)

Season	<i>A. fumigatus</i>	Grass pollen	Horse dander	<i>D. pteronyssinus</i>
Autumn	-0.04	0.47***	0.07	0.55***
Winter	0.01	0.50***	0.21	0.45***
Spring	0.14	0.39***	0.03**	0.53***
Summer	0.12	0.61***	0.28**	0.52***
Autumn	0.19*	0.57***	0.17	0.51***
Patients positive (range)	7-11	16-19	18-21	19-20

* $P < 0.05$.** $P < 0.01$ *** $P < 0.001$ **Table 4.** IgG antibody to *D. pteronyssinus* allergen related to weal size produced by the same allergen

Weal size to <i>D. pteronyssinus</i> antigen over 1 year	Presence (+) or absence (-) of IgG antibody to <i>D. pteronyssinus</i> allergen at the start and end of 1 year of study							
	+	+	-	-	+	-	-	+
	(12)		(15)		(3)		(5)	
Bigger	1		1		1		0	
Smaller	6		8		0		2	
Negative	5		5		2		3	
Unchanged	0		1		0		0	

highly significant ($W=0.85$, $P \leq 0.001$) for both grass pollen and *D. pteronyssinus*, demonstrating a consistency in ranking between the patients.

Specific IgG

Fifteen patients had no IgG to *D. pteronyssinus* at the start or the end of the period looked at. Twelve had IgG at the start and at the end, five developed it and three lost it. Table 4 shows the relationship of skin test size to the presence, absence, development of and loss of antibody to *D. pteronyssinus* during this period. IgG antibody to *D. pteronyssinus* did not appear to affect weal size.

Discussion

This study showed that when prick skin tests are performed on the volar aspect of the forearm away from the wrist and antecubital fossa as recommended by Hendrick *et al.* (1975), the weal size is not altered by varying the position of testing within this area.

When the tests were repeated over a period of time, different reactions were

obtained with the various allergens. For *A. fumigatus*, grass pollen and horse dander, a significant variation in the percentage of patients with positive skin tests occurred. For horse dander, a significant decrease in the number of positive reactions occurred from Autumn 1973 to Autumn 1975. This would suggest that a single prick skin test result, if negative, should not be relied upon if one wishes to detect sensitivity to an allergen, e.g. screening workers for the development of sensitivity to compounds in industry. In a study in asthmatic children (mean age 8.6 years), all having positive bronchial provocation tests to *D. pteronyssinus*, prick skin tests to a range of common allergens were repeated after an interval of 6 months. Reactions were gained and lost to the different allergens over this period and this was related to worsening or improvement in asthmatic symptoms (Warner, 1977).

A variation in weal size also occurred for all allergens except *C. albicans*; this variation was found to be associated with a significant reduction in weal size over time for grass pollen, horse dander and *D. pteronyssinus*. For horse dander, there was therefore a significant reduction in the number of patients with positive reactions and a significant reduction in the size of the reactions remaining over the course of the study.

These variations require explanation. No attempt was made to relate the skin test reactions to the state of the patient's asthma as was done by Warner (1977), so the explanation presented in that study cannot be commented upon. The same allergen batches were used throughout the study and stored as recommended by the manufacturer, so variation in allergen quality cannot be incriminated. It is possible that repeated prick skin testing or repeated natural exposure to the allergens over time could have caused the production of IgG antibodies; they in turn may have interfered with the reaction taking place on the mast cell following prick skin testing, thus reducing the weal size or preventing it developing. IgG antibody to *D. pteronyssinus* was looked for in these patients but no relationship could be found between antibody and weal size. Hyposensitization was shown in one study to have no effect on skin test reactions (Kjellman *et al.*, 1977).

Another possible explanation for the reduction in weal size with time is the effect of age on the reaction. It has been reported that ageing causes a reduction in the ability to produce an IgE mediated Type I reaction and a reduction in serum IgE concentration (Orren & Dowdle, 1975; Delespesse *et al.*, 1977; Hanneuse *et al.*, 1978). Our method of testing these patients may have detected this decline in immunological reactivity to this antibody system. It would appear that ageing does not effect the skin reaction induced by histamine itself (Delespesse *et al.*, 1977).

Specific IgE estimations were determined for four allergens over five seasons: grass pollen, horse dander, *D. pteronyssinus* and *A. fumigatus*. The allergens used were the same as those used for skin testing. A seasonal variation in IgE to grass pollen and *D. pteronyssinus* was demonstrated, but there was no fall off in these levels over the period as was noted for weal size to the same allergens. A previous study has also demonstrated a seasonal variation in IgE to grass pollen (Berg & Johansson, 1971). The failure to show a decline in specific IgE levels to these two allergens would suggest that the decline in weal size is not related to serum IgE levels and would support a loss of sensitivity on the part of the patient to this reaction.

Nevertheless, with two of the allergens, grass pollen and *D. pteronyssinus*, a good correlation between specific IgE levels and weal size was demonstrated. There was poor correlation between specific IgE and weal size for *A. fumigatus* and horse dander. This

may have been due to the quality of these allergens, a factor known to be critical in this situation (Aas, 1975).

Other mechanisms for this latter poor correlation include failure to couple the optimal concentration of allergen on the discs for RAST testing (Baldo, Quinn & Turner, 1975; Editorial, 1977); the skin reactions to these allergens were in some instances toxic or non-specific reactions due to contaminants in the allergen mixtures (Guerin & Tioulong, 1979) and some of the skin reactions may have been mediated by IgG short-term sensitizing antibody (Parish, 1970) or due to direct stimulation of complement components (Berrens, 1974). The RAST results, it must be remembered, are a measurement of circulating specific IgE and this may not reflect the amount of IgE fixed to receptors on tissue mast cells and capable of reacting in skin tests. The consistency in the specific IgE results for each allergen within patients over the period of measurement was very high.

This study suggests that repeated prick skin testing may be a way of monitoring the reactivity of the IgE system. The repeatability of prick skin test reactions when the volar aspect of the forearm would allow this method to be used to measure the patient's reactivity to various allergens.

References

- AAS, K. (1975) Clinical and experimental aspects of standardization and purification of allergens. *International Archives of Allergy and Applied Immunology*, **49**, 44.
- BALDO, B.A., QUINN, E.H. & TURNER, K.J. (1975) *In vitro* diagnosis of allergy: radio allerge sorbent test (RAST) studies with some allergens commonly encountered in Australia. *Medical Journal of Australia*, **2**, 859.
- BERG, T. & JOHANSSON, S.G.O. (1971) *In vitro* diagnosis of atopic allergy. IV: seasonal variations of IgE antibodies in children allergic to pollens: a study of non-treated children and of children treated with inhalation of disodium cromoglycate. *International Archives of Allergy*, **41**, 452.
- BERRENS, L. (1974) Inhalant allergens in human atopic disease: their chemistry and mode of action. *Annals of the New York Academy of Sciences*, **221**, 183.
- DELESPESE, E., DE MAUBEUGE, J., KENNES, B., NICAISE, R. & GOVAERTS, A. (1977) IgE mediated hypersensitivity in ageing. *Clinical Allergy*, **7**, 65.
- EDITORIAL (1977) Diagnosis of Allergy. *British Medical Journal*, **1**, 468.
- GUERIN, B. & TIOULONG, S. (1979) Analysis of the non-immunological activity of allergen extracts in cutaneous tests. *Clinical Allergy*, **9**, 283.
- HANNEUSE, V., DELESPESE, G., HUDSON, D., DEHALLEUX, I. & JACQUES, J.M. (1978) Influence of ageing on IgE mediated reactions in allergic patients. *Clinical Allergy*, **8**, 165.
- HENDRICK, D.J., DAVIES, R.J., D'SOUZA, M.F. & PEPYS, J. (1975) An analysis of skin prick test reactions in 656 asthmatic patients. *Thorax*, **30**, 2.
- KJELLMAN, N., KJELLMAN, I.M., LANNER, Å. & ROTH, A. (1977) Predictive value of serum IgE levels during rush hyposensitization. *Clinical Allergy*, **7**, 465.
- MCALLEN, M.K., ASSEM, E.S.K. & MAUNSELL, K. (1970) House dust mite in asthma: results of challenge tests on five criteria with *Dermatophagoides pteronyssinus*. *British Medical Journal*, **2**, 501.
- ORREN, A. & DOWDLE, B.B. (1975) The effects of sex and age on serum IgE concentration in three asthmatic groups. *International Archives of Allergy and Applied Immunology*, **48**, 824.
- PARISH, W.E. (1970) Short-term anaphylactic IgG antibodies in human sera. *Lancet*, **i**, 591.
- PEPYS, J. (1973) Types of allergic reaction. *Clinical Allergy*, **3**, Suppl., 491.
- PEPYS, J., CHAN, M. & HARGREAVE, F.E. (1968) Mites and house-dust allergy. *Lancet*, **i**, 1270.
- PEPYS, J., WELLS, I.D., D'SOUZA, M.F. & GREENBERG, M. (1973) Clinical and immunological responses to enzymes of *Bacillus subtilis* in factory workers and consumers. *Clinical Allergy*, **3**, 143.
- STENIUS, W., WIDE, L., SEYMOUR, W.M., HOLFORD-STREVEN, V. & PEPYS, J. (1971) Clinical significance of specific IgE to common allergens. *Clinical Allergy*, **1**, 37.
- TURNER-WARWICK, M. (1973) Immunology of the respiratory tract. In: *Recent Advances in Medicine*, eds, D. N. Baron, N. Compston and A. M. Dawson, 16th edition, p. 143. Churchill Livingstone, Edinburgh.

- WARNER, J.O. (1977) The variability of skin hypersensitivity reactions in cystic fibrosis and asthma. *Clinical Allergy*, **7**, 385.
- WIDE, L., BENNICH, H. & JOHANSSON, S.G.O. (1967) Diagnosis of allergy in an *in vitro* test for allergen antibodies. *Lancet*, **ii**, 1105.
- ZETTERSTROM, O. & WIDE, L. (1974) IgE antibodies and skin test reactions to a detergent-enzyme in Swedish consumers. *Clinical Allergy*, **4**, 273.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.